ing Spectrophotometer whose wave length and optical density scales had been calibrated.

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Paper Chromatography of Bile Acids[1]

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The separation of bile acids by paper chromatography has been investigated in this Laboratory and two solvent systems which give different, reproducible R_f values for several acids have been found. The two systems are *n*-propyl alcohol–ammonia–water 90:2:8 and *n*-propyl alcohol–ethanolamine–water 90:5:5. Of the two systems, the latter concentrates the moving material into a smaller area and is, therefore, preferable for identification or separation.

Using this solvent mixture, we have been able to achieve separation of various mixtures of these bile acids. Although the R_f values of dehydrocholic, cholic and norcholic acids are close together, we have been able to separate mixtures of desoxycholic, dehydrocholic and cholic acids, and of desoxycholic, dehydrocholic and norcholic acid. In these experiments we have generally observed two distinct spots of the two acids whose R_f values are close together; in some cases, however, they merge to give one spot. All experiments were carried out using 50 γ of material; mixtures contained 50 γ of each component.

For identification of the bile acids, a 15% phosphoric acid spray, slightly different from that originally proposed by Neher and Wettstein,[2] was used. The acids appeared as brown or red spots in white light, or displayed a greenish-yellow or pink fluoresence in ultraviolet light.

The results are given in Tables I and II.

TABLE I

R_f VALUES FOR BILE ACIDS

Acid	P–M–W[a] 90:5:5	P–A–W 90:2:8	E–A–W 90:2:8	P–A–W 5:2:3
Desoxycholic	0.92	0.74	0.66	0.95
Dehydrocholic	.65	.47	.65	.89
Cholic	.71	.52	.71	.94
Norcholic	.69	.51	.70	.94
Triformylnorcholic	.92	.68	.75	.94

[a] P, *n*-propyl alcohol; M, monoethanolamine; A, ammonia; W, water.

TABLE II

SEPARATIONS

Mixture	R_f values
Desoxycholic/dehydrocholic/cholic	0.95/0.65/0.72
Desoxycholic/dehydrocholic/norcholic	0.92/0.62/0.73

Experimental

The organic solvents were distilled prior to use. All mixtures are by volume as given. Whatman #1 paper was used throughout.

The material to be chromatographed was applied to a spot about 2 cm. in diameter on a 4 × 40-cm. strip of filter paper. Descending chromatography was used and after the solvent front had advanced 25–35 cm. from the origin, the strips were removed from the chromatographic chamber (a 7 × 50-cm. test-tube) and air-dried. Prior to spraying, the strips were dried at 80° for 15 minutes. The spray solution was prepared by mixing 10 parts of 85% phosphoric acid with 25 parts each of water and 95% ethanol. After the papers were sprayed, they were kept at 90° for 20 minutes. Generally, cholic and norcholic acids showed up as red or brick colored spots and occasionally one of the other acids appeared as a red spot. In ultraviolet light (Model SL Mineralight, Ultra-Violet Products, Inc., South Pasadena, California) desoxycholic acid exhibited a pink fluorescence and the other acids exhibited a greenish-yellow fluorescence. When larger quantities of these acids were used (100–200 γ) they all gave colored spots in white light as well as appearing more readily in the ultraviolet.

The R_f values were measured from the foremost point of the origin to the leading edge of the spot. The solvent mixtures which included ammonia tended to give some streaking, whereas with ethanolamine spots about 15 mm. in diameter were obtained.

All R_f values represent the average of a number of experiments.

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(1) The work described in this paper was sponsored by the United States Atomic Energy Commission.

(2) R. Neher and A. Wettstein, *Helv. Chim. Acta*, **34**, 2278 (1951).

A Redetermination of the Kinetic Constants for the System α-Chymotrypsin–Nicotinyl-L-tryptophanamide[1]

BY H. T. HUANG AND CARL NIEMANN[2]

RECEIVED MARCH 29, 1952

In previous studies of the kinetics of the α-chymotrypsin catalyzed hydrolysis of simple specific substrates[3–8] the enzyme preparations used were obtained from a single source, *i.e.*, Armour and Co., although it is true that care was taken to use preparations of different lot numbers in several of the investigations.[4,6] While it has been possible in one instance[4] to compare the K_S and k_3 values of acetyl L-tyrosinamide obtained in these laboratories with those obtained elsewhere[9–13] with different enzyme preparations the fact that differences in the reaction systems and analytical procedures were also involved in the above comparison suggested the desirability of a comparison in which the source of the enzyme preparation was the only variable.

The Armour preparation used most frequently in our previous investigations bore the lot no. 90402. This preparation had been used at three different concentrations in a total of twenty-eight separate experiments to evaluate the

(1) Supported in part by a grant from Eli Lilly and Co.

(2) To whom inquiries regarding this article should be sent.

(3) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).

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(9) S. Kaufman and H. Neurath, *Arch. Biochem.*, **21**, 245 (1949).

(10) S. Kaufman and H. Neurath, *J. Biol. Chem.*, **180**, 181 (1949).

(11) G. W. Schwert and S. Kaufman, *ibid.*, **180**, 517 (1949).

(12) S. Kaufman and H. Neurath, *ibid.*, **181**, 623 (1949).

(13) H. Neurath and J. A. Gladner, *ibid.*, **188**, 407 (1951).

K_S and k_3 values of nicotinyl-L-tryptophanamide at 25° and *p*H 7.9 in aqueous systems 0.02 *M* in respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer.[3] Thus with these relatively precise values at hand and also because this specific substrate had been used in inhibition experiments to evaluate the K_I values of a large number of competitive inhibitors[14] it was selected for use in this investigation. For comparison with the above Armour preparation a sample of α-chymotrypsin originally obtained as a twice recrystallized filter cake from the Worthington Biochemical Laboratories, which had been further recrystallized and then dialyzed in the cold, first against dilute aqueous hydrochloric acid of *p*H 3.5, then exhaustively against water, and finally lyophilized, was kindly placed at our disposal by Mr. E. F. Jansen of the Western Regional Research Laboratory. The experimental conditions and the analytical methods used in this investigation were identical with those employed previously.[3]

The results of the present study are summarized in Fig. 1 wherein eight determinations of the initial velocity at seven different initial substrate concentrations and a single enzyme concentration are expressed as a $[S]_0$ *versus* $[S]_0/v_0$ plot.[15] From the intercept of this plot, *i.e.*, $-K_S$, and the slope, *i.e.*, V, the K_S and k_3 values for nicotinyl-L-tryptophanamide were found to be 2.7×10^{-3} *M* and 1.5×10^{-3} mole/liter/min./mg. protein-nitrogen/ml., respectively.[16] These values are in excellent agreement with the previously determined K_S and k_3 values of 2.7×10^{-3} *M* and 1.6×10^{-3} mole/liter/min./mg. protein-nitrogen/ml., respectively.[3]

It can be concluded from the above results that the kinetic constants reported previously for systems containing bovine α-chymotrypsin[3-8,14] are those of systems containing a reproducible characteristic catalytic species of considerable stability. However, the agreement noted above adds little to what is already known about the accuracy of these values,[3] since an error that can still be present is the operational one involved in the determination of initial velocities and this has in a sense been standardized by using approximately the same procedure in all cases. An investigation is now in progress in which it is hoped that initial velocities can be estimated with much greater accuracy than has previously been possible.

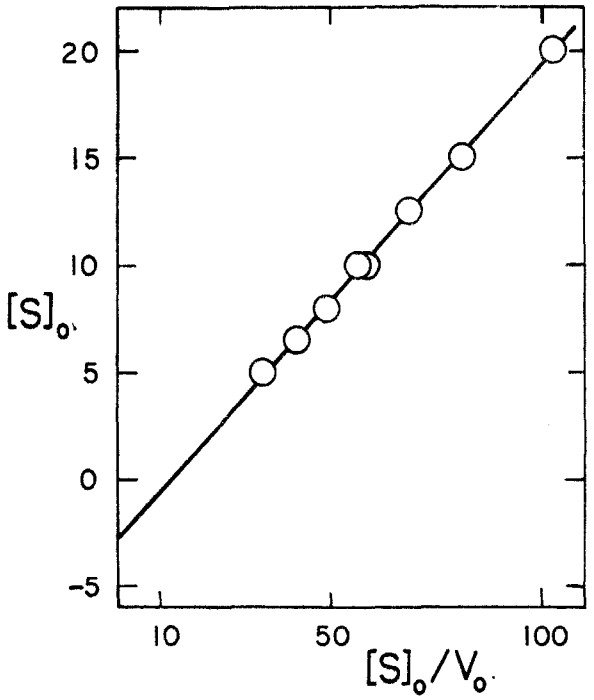

Fig. 1.—α-Chymotrypsin catalyzed hydrolysis of nicotinyl-L-tryptophanamide at 25° and *p*H 7.9; v_3 in units of 10^{-3} *M* per min., $[S]_0$ in units of 10^{-3} *M*, [E] equivalent to 0.144 mg. protein–nitrogen per ml., 0.02 *M* tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer.

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(16) An independent evaluation of these data by Dr. D. W. Thomas and based upon a least squares treatment gave $K_S = 2.8 \times 10^{-3}$ *M* and $k_3 = 1.5 \times 10^{-3}$ mole/liter/min./mg. protein-nitrogen/ml.

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Anion Exchange of Niobium in 7.0 Molar Hydrochloric Acid

BY E. H. HUFFMAN AND G. M. IDDINGS

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It has recently[1] been reported that the elution of titanium from a cation exchange resin with citrate solution has resulted in broad elution bands with several peaks. This behavior was attributed to the probable partial separation of the isotopes of titanium. Work in this Laboratory on the elution of niobium with hydrochloric acid from an anion exchange resin, subsequent to that previously reported,[2] has shown a somewhat similar behavior, but under conditions which precluded any possible isotope separation.

When carrier-free Nb^{95}, prepared as described before,[2] was adsorbed from a 10.0 *M* hydrochloric acid solution on a Dowex 2 anion exchange resin column, 8.0 cm. long and 3.0 mm. in diameter, and then eluted with 7.0 *M* hydrochloric acid at the rate of about 2.4 ml. per hour, the elution curve shown in Fig. 1 was obtained. The possibility of any foreign activity in the purified Nb^{95} accounting for three peaks was eliminated by obtaining the decay rates of the samples taken at the top of each

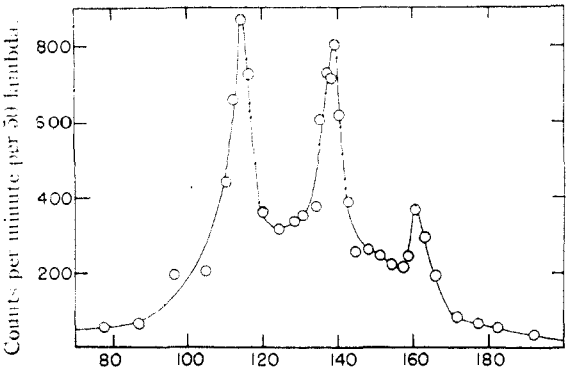

Fig. 1.

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